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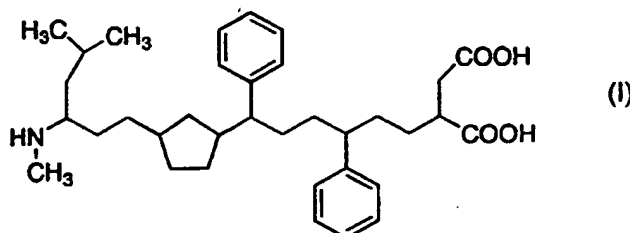
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(54) Title: PEPTIDE ANALOGS AND MIMETICS SUITABLE FOR IN VIVO USE IN THE TREATMENT OF DISEASES ASSOCIATED WITH ABNORMAL PROTEIN FOLDING INTO AMYLOID, AMYLOID-LIKE DEPOSITS OR β -SHEET RICH PATHOLOGICAL PRECURSOR THEREOF



(57) Abstract: The present invention is an inhibitory peptide capable of inhibiting β pleated sheet formation in amyloid β -peptide. The inhibitory peptide is a β sheet breaker peptide analog designed by chemical modification of β sheet breaker peptide capable of inhibiting β pleated sheet formation in amyloid β -peptide. The present invention also includes an inhibitory peptide capable of inhibiting conformational changes in prion PrP protein associated with amyloidosis. The inhibitory peptide being a β sheet breaker peptide analog designed by chemical modification of a β sheet breaker peptide capable of inhibiting the conformational changes in prior PrP protein associated with amyloidosis. In addition, the present invention includes a peptide mimetic with the structure PMiA β 5. In another embodiment, the peptide mimetic has the structure PMiPrP13. In yet another embodiment, the peptide mimetic has the structure PMiPrP5.

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**PEPTIDE ANALOGS AND MIMETICS SUITABLE FOR IN VIVO USE IN THE
TREATMENT OF DISEASES ASSOCIATED WITH ABNORMAL PROTEIN
FOLDING INTO AMYLOID, AMYLOID-LIKE DEPOSITS OR β -SHEET RICH
PATHOLOGICAL PRECURSOR THEREOF**

This application claims priority from U.S. Provisional Application No. 60/163,911,
which was filed on November 5, 1999.

5 BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to peptide analogs and peptide mimetics of β -sheet
breaker peptides suitable for *in vivo* use in treating mammals with protein conformational
diseases such as Alzheimer's and prion disease. More particularly, the present invention is
10 directed to novel peptide analogs and mimetics, pharmaceutical compositions containing one
or a mixture of such peptide analogs and mimetics, and methods for preventing, treating, or
detecting disorders or diseases associated with abnormal protein folding into amyloid or
amyloid-like deposits or precursors thereof having a pathological beta-sheet structure.

15 Description of Related Art

Extensive evidence has been accumulated indicating that several diverse disorders
have the same molecular basis, i.e. a change in a protein conformation (Thomas et al.,
Trends Biochem. Sci. 20: 456-459, 1995; Soto, *J. Mol. Med.* 77: 412-418, 1999). These
protein conformational diseases include Alzheimer's disease, prion-related disorders,

systemic amyloidosis, serpin-deficiency disorders, Huntington's disease and Amyotrophic Lateral Sclerosis (Soto 1999, supra). The hallmark event in protein conformational disorders is a change in the secondary and tertiary structure of a normal protein without alteration of the primary structure. The conformationally modified protein may be implicated in the disease by direct toxic activity, by the lack of biological function of normally-folded protein, or by improper trafficking (Thomas et al., 1995, supra). In the cases where the protein is toxic, it usually self-associates and becomes deposited as amyloid fibrils in diverse organs, inducing tissue damage (Thomas et al., 1995, supra; Kelly, *Curr. Opin. Struct. Biol.* 6: 11-17, 1996; Soto, 1999, supra).

Alzheimer's disease (AD) is a devastating neurodegenerative problem characterized by loss of short-term memory, disorientation, and impairment of judgment and reasoning. AD is the most common dementia in elderly population. It is estimated that more than twenty-five million people worldwide are affected in some degree by AD (Teplow, *Amyloid* 5: 121-142, 1998). A hallmark event in AD is the deposition of insoluble protein aggregates, known as amyloid, in brain parenchyma and cerebral vessel walls. The main component of amyloid is a 4.3 KDa hydrophobic peptide, named amyloid beta-peptide (A β) that is encoded on the chromosome 21 as part of a much longer precursor protein (APP) (Selkoe, *Science* 275: 630-631, 1997). Genetic, biochemical, and neuropathological evidence accumulated in the last 10 years strongly suggest that amyloid plays an important role in early pathogenesis of AD and perhaps triggers the disease (Soto et al., *J. Neurochem.* 63: 1191-1198, 1994; Selkoe, 1997, supra; Teplow, 1998, supra; Sisodia and Price, *FASEB J.* 9:

366-370, 1995; Soto, *Mol. Med. Today* 5: 343-350, 1999).

Amyloid is a generic term that describes fibrillar aggregates that have a common structural motif, i.e., the β -pleated sheet conformation (Serpell et al., *Cell Mol. Life Sci.* 53: 887, 1997; Sipe, *Ann. Rev. Biochem.* 61: 947-975, 1992). These aggregates exhibit specific tinctorial properties, including the ability to emit a green birefringent glow after staining with Congo red, and the capacity to bind the fluorochrome, thioflavin S (Sipe, 1992, supra; Ghiso et al., *Mol. Neurobiol.* 8: 49-64, 1994). There are more than a dozen human diseases of different etiology characterized by the extracellular deposition of amyloid in diverse tissues, which lead to cell damage, organ dysfunction, and death. Among the diseases involving amyloidosis, it is possible to highlight Alzheimer's disease, prion-related disorders (also known as transmissible spongiform encephalopathy), and systemic amyloidosis (Table 1). The amyloid fibrils are usually composed of proteolytic fragments of normal or mutant gene products. There are over 16 different proteins (Table 1) involved in amyloid deposition in distinct tissues (Ghiso et al., 1994, supra).

The formation of amyloid is basically a problem of protein folding, whereby a mainly random coil soluble peptide becomes aggregated, adopting a β -pleated sheet conformation (Kelly, 1996, supra; Soto, 1999, supra). Amyloid formation proceeds by hydrophobic interactions among conformationally altered amyloidogenic intermediates, which become structurally organized into a β -sheet conformation upon peptide interaction. The hydrophobicity appears to be important to induce interaction of the monomers leading to aggregation, while the β -sheet conformation might determine the ordering of the aggregates

in amyloid fibrils. In an attempt to inhibit amyloid fibril formation, these two properties were separated by designing short synthetic peptides bearing sequence homology and a similar degree of hydrophobicity as the peptide domain implicated in the conformational change, but having a very low propensity to adopt a β -sheet conformation (called β -sheet breaker peptides) (Soto et al., 1996, supra; Soto et al., 1998, supra). The aim was to design a peptide with the ability to bind specifically to the amyloidogenic peptide forming a complex that stabilizes the physiological conformation and destabilizes the abnormal conformation of the peptide (Soto, 1999, supra).

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Table 1. Disorders related with amyloidosis and the protein component of the amyloid fibrils

DISEASE	FIBRIL COMPONENT
Alzheimer's disease	Amyloid- β protein
Primary systemic amyloidosis	Immunoglobulin light chain or fragments thereof
Secondary systemic amyloidosis, Familial Mediterranean fever	Fragments of serum amyloid-A
Spongiform encephalopathy	Fragments of prion protein
Senile systemic amyloidosis, Familial amyloid polyneuropathy	Transthyretin and fragments thereof
Hemodialysis-related amyloidosis	β 2-microglobulin
Hereditary cerebral amyloid angiopathy, Icelandic type	Cystatin C
Familial amyloidosis, Finnish type	Gelsolin fragments
Type II diabetes	Fragments of islet amyloid polypeptide
Familial amyloid polyneuropathy	Fragments of apolipoprotein A-I
Medullar carcinoma of the thyroid	Fragments of calcitonin
Atrial amyloidosis	Atrial natriuretic factor
Hereditary non-neuropathic systemic amyloidosis	Lysozyme or fragments thereof
Hereditary renal amyloidosis	Fibrinogen fragments
Islet amyloid	Insulin
Amyloidosis in senescence	Apolipoprotein A-II

β -sheet breaker peptides have so far been designed to block the conformational changes that occur in both A β and prion protein (PrP), which are implicated in the pathogenesis of Alzheimer's and prion disease, respectively. The prior art has previously shown that 11- and 5-residue β -sheet breaker peptides (namely, iA β 1 and iA β 5, respectively) homologous to the central hydrophobic region of A β inhibit peptide conformational changes that result in amyloid formation and also dissolved preformed fibrils *in vitro* (Soto et al., *Biochem. Biophys. Res. Commun.* 226: 672-680, 1996; Soto et al., *Nature Med.* 4: 822-826, 1998). In addition, the 5-residue peptide is capable of preventing the neuronal death induced by the formation of β -sheet rich oligomeric A β structures in cell culture experiments (Soto et al., 1998, supra). Furthermore, by using a rat model of amyloidosis induced by intracerebral injection of A β 1-42, the prior art has shown that co-injections of the 5-residue β -sheet breaker peptide decreased cerebral A β accumulation and completely blocked the deposition of fibrillar amyloid-like lesions in the rat brain (Soto et al., 1998, supra). Finally, the β -sheet breaker peptide injected eight days after the injection of A β was able to disassemble preformed A β fibrils in the rat brain *in vivo*, that leads to a reduction in the size of amyloid deposits (Sigurdsson, Frangione, Soto, manuscript submitted). Interestingly, removal of amyloid by the β -sheet breaker peptide reverts the associated cerebral histologic damage, including neuronal shrinkage and microglial activation.

β -sheet breaker peptides have also been designed to prevent and to revert conformational changes caused by prions (PrP). Based on the same principles and using as a template the PrP sequence 114-122, the prior art has shown that when a set of β -sheet

breaker peptides was synthesized, a 13-residue peptide (iPrP13) showed the greatest activity (Soto, 1999, *supra*). Several *in vitro* cell culture and *in vivo* assays were used to test for inhibitory activity and the results clearly indicated that it is possible not only to prevent the PrP^c → PrP^{sc} conversion, but more interestingly to revert the infectious PrP^{sc} conformer to a
5 biochemical and structural state similar to PrP^c (Soto et al., manuscript submitted).

Short peptides have been utilized extensively as drugs in medicine (Rao et al., C. Basava and G.M. Anantharamaiah, eds. *Boston: Birkhauser*, pp. 181-198, 1994). However, the development of peptide drugs is strongly limited by their lack of oral bioavailability and their short duration of action resulting from enzymatic degradation *in vivo* (Fauchere and
10 Thurieau, *Adv. Drug Res.* 23: 127-159, 1992). Progress in recent years toward the production of peptide analogs (such as pseudopeptides and peptide mimetics) with lower susceptibility to proteolysis has increased the probability to obtain useful drugs structurally related to their parent peptides (Fauchere and Thurieau, 1992, *supra*). Improving peptide stability to proteases not only increases the half-life of the compound in the circulation but
15 also enhances its ability to be transported or absorbed at different levels, including intestinal absorption and blood-brain barrier permeability, because transport and absorption appear to be highly dependent upon the time of exposure of membranes or barriers to the bioactive species (Fauchere and Thurieau, 1992, *supra*).

20 SUMMARY OF THE INVENTION

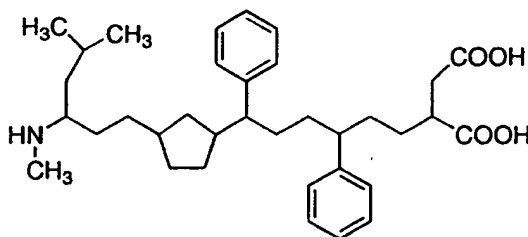
The present invention is an inhibitory peptide capable of inhibiting β pleated sheet

formation in amyloid β -peptide, the inhibitory peptide being a β sheet breaker peptide analog designed by chemical modification of a β sheet breaker peptide capable of inhibiting β pleated sheet formation in amyloid β -peptide.

The peptide is altered chemically by: (1) modifications to the N- and C-terminal ends of the peptide; (2) changes of the side-chain, which can involve amino acid substitutions; (3) modification in the α -carbon including methylations, alkylations and dehydrogenations; (4) chirality changes by replacing D- for L-residue; (5) head-to-tail cyclizations; and (6) introduction of amide bond replacements, i.e. changing the atoms participating in the peptide (or amide) bond.

The present invention also includes an inhibitory peptide capable of inhibiting conformational changes in prion PrP protein associated with amyloidosis, the inhibitory peptide being a β sheet breaker peptide analog designed by chemical modification of a β sheet breaker peptide capable inhibiting the conformational changes in prior PrP protein associated with amyloidosis.

In addition, the present invention includes a peptide mimetic with the following structure:

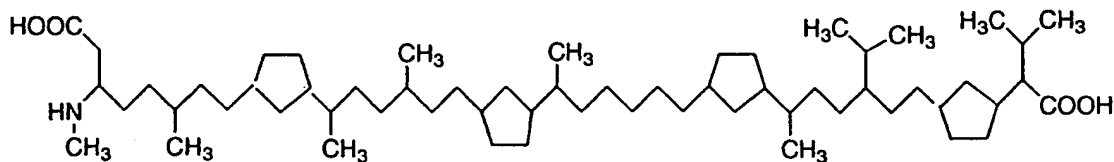




PMiA85

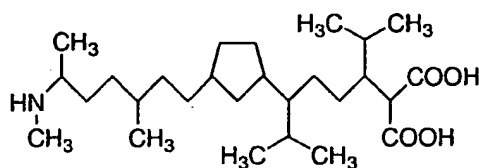


In another embodiment, the peptide mimetic has the following structure:



PMiPrP13

5 In yet another embodiment, the peptide mimetic has the following structure:



PMiPrP5

The present invention also includes a method for preventing, treating, or detecting

10 disorders or diseases associated with abnormal protein folding into amyloid or amyloid-like deposits or precursors thereof having a pathological beta-sheet structure is claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic representation of the peptide bond and the potential target sites

15 for peptide modifications.

FIG. 2 is a graph depicting the pharmacokinetics of a 11-residue β -sheet breaker peptide inhibitor of Alzheimer's amyloidosis (Seq. RDLPFYPVPID) in its natural L-configuration and in the non-natural D-form.

FIGS. 3A and 3B are representations of the tridimensional structure of Alzheimer's and prion β -sheet breaker peptides iA β 5 and iPr P13, respectively.

FIG. 4a and 4b are graphs showing the bioavailability and stability of iA β 5 and Ac-iA β 5-Am, respectively over time.

FIG. 5a provides a graphical comparison of A β 1-40 incubated with various other peptides.

FIG. 5b is a graph of amyloid formation vs. the Ac-iA β 5-Am concentration.

FIG. 5c is a graph of amyloid formation vs. the iA β 5 concentration.

FIG. 6 shows a model where there is an 83% dissolution of deposits in the ventricle area and a 30% dissolution of amyloid plaque in the amygdala.

DETAILED DESCRIPTION OF THE INVENTION

In the present invention, the bioavailability and stability of an inhibitory peptide is improved by chemically modifying the parent peptide to produce a derivative more suitable for *in vivo* use, which is preferably administered orally. The inhibitory peptide is capable of inhibiting β pleated sheet formation in an amyloid β -peptide. Moreover, the inhibitory peptide is a β sheet breaker peptide analog designed by chemical modification of a β sheet breaker peptide capable of inhibiting β pleated sheet formation in amyloid β -peptide.

This invention also includes an inhibitory peptide capable of inhibiting conformational changes in prion PrP protein associated with amyloidosis, where the inhibitory peptide is a β sheet breaker peptide analog designed by chemical modification of a β sheet breaker peptide and is capable of inhibiting the conformational changes in prion PrP protein associated with amyloidosis.

In Fig. 1, a generalized peptide backbone is shown, where possible targets for chemical modification are highlighted. The possible targets include the following: (1) modifications to the N- and C-terminal ends of the peptide (targets a and b); (2) changes of the side-chain (target c) which usually involve amino acid substitutions; (3) modification in the α -carbon (target d) including methylations, alkylations and dehydrogenations; (4) chirality changes by replacing D- for L-residue; (5) head-to-tail cyclizations; and (6) introduction of amide bond replacements (target e), i.e. changing the atoms participating in the peptide (or amide) bond. The latter derivatives are known as pseudopeptides or amide bond surrogates.

Natural peptides are usually degraded by the concerted action of specialized endopeptidases and unspecific exopeptidases. Endopeptidases are often present in tissues and cellular compartments and convert the peptide into two or more inactive fragments. Exopeptidases are generally present in blood and peripheral organs and carry out the degradation of the intact peptides or their fragments to the constituent amino acids and hence contribute to the disappearance of the peptides from the circulation. Exopeptidases recognize the free amino or carboxyl groups in peptides. Therefore, modification of those

groups often diminish or abolish exopeptidase degradation. Head-to-tail peptide cyclization results in the absence of free end-terminal groups, and hence also minimizes cleavage by exopeptidases. On the other hand, endopeptidases recognize the atoms participating in the amide bond. Thus, amide bond replacements dramatically decrease degradation by endopeptidases. The same usually happens with modifications to the α -carbon. Since most (if not all) of the exo- and endo-proteases are stereospecific, substitutions of the natural L-amino acids by the D-stereoisomers result in a clear increase in peptide stability. Finally, peptide mimetics are usually completely resistant to proteolytic degradation and often can be administered orally.

β -sheet breaker analogues designed by chemical modifications of the lead peptides.

Starting from the 5-residue Alzheimer's inhibitor peptide (iA β 5, Seq. LPFFD – also denoted as Leu Pro Phe Phe Asp) and the 13-residue prion inhibitor peptide (iPrP13, Seq. DAPAAPAGPAVPV – also denoted as Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro Val), the modifications described below are designed. The peptides used in the present invention are synthesized using standard protocols as disclosed by Bergmann et al., and incorporated herein by reference. (Bergmann & Zervas, Berichte der Deutschen Chemischen Gesellschaft (1932) 65: 1192 – 1201)

a) *N- and C-terminal modifications.* N-terminal acetylation or desamination confers protection against digestion by a number of aminopeptidases while the presence of amides or alcohols replacing the C-terminal carboxyl group prevent splitting by several

carboxypeptidases, including carboxypeptidases A and B. The altered peptide sequences including these modifications are the following, where ac is acetylation, am is amidation, des is desamination, and alc is alcoholization:

Alzheimer's InhibitorsPrion Inhibitors

5	ac-Leu Pro Phe Phe Asp-am	ac-Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro Val-am
	des-Leu Pro Phe Phe Asp-am	des-Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro Val-am
	ac-Leu Pro Phe Phe Asp-alc	ac-Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro Val-alc
	des-Leu Pro Phe Phe Asp-alc	des-Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro Val-alc

10 b) *Side-chain changes*. The presence of non-natural amino acids usually increase peptide stability. In addition, at least one of these amino acids (α -aminoisobutyric acid or Aib) imposes significant constraints to model peptides diminishing their conformational flexibility. In particular, the incorporation of Aib into β -sheet model peptides induces the complete disruption of this structure. The β -sheet blocking activity of Aib is comparable or
 15 even greater than the natural residue proline used in the peptide as a β -sheet blocker. Therefore, the introduction of Aib is expected to enhance peptide stability and inhibitory activity at the same time.

Alzheimer's InhibitorsPrion inhibitors

Leu Aib Phe Phe Asp Asp Ala Aib Ala Ala Aib Ala Ala Aib Ala Gly Aib Ala Val Aib Val

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c) *Modifications in the α -carbon*. The most commonly used α -carbon modification to improve peptide stability is α -methylation. In addition, replacement of the hydrogen atom

linked to the α -carbon of Phe, Val or Leu has been shown to favor the adoption of β -bend conformation and strongly disfavor the formation of β -pleated sheet structures. According to the present invention, methylation of those residues in the inhibitor peptides is expected to enhance stability and potency.

5 Alzheimer's Inhibitors

(Me)Leu Pro Phe Phe Asp

Leu Pro (Me)Phe Phe Asp

Leu Pro Phe (Me)Phe Asp

(Me)Leu Pro (Me)Phe (Me)Phe Asp

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Prion inhibitors

Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala (Me)Val Pro Val

Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro (Me)Val

Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala (Me)Val Pro (Me)Val

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d) *Chirality changes.* Replacement of the natural L-residue by the D-enantiomers dramatically increases resistance to proteolytic degradation. The increase in stability by introduction of D-residue has already been demonstrated for the 11-residue β -sheet breaker peptide (iA β 1). *In vivo* studies showed that the peptide bearing the natural sequence rapidly degraded in rat plasma. Indeed, approximately 90% of iA β 1 was degraded within minutes after intravenous injection. Conversely, a derivative of iA β 1 containing all the residue in the D-form showed virtually no degradation in the plasma after injection for 15 minutes. For

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detection, the peptide was radio-iodinated using standard procedures. Peptide stability was evaluated after i.v bolus injection in rats by precipitation with trichloroacetic acid.

Quantitation of the intact peptide was also done by paper chromatography. Thus, iA85 and iPrP13 peptides (FIGS. 3A and 3B, respectively) containing all-D residue as well as

peptides containing D-residue only at the N- and C-terminal ends to prevent exopeptidase degradation are included in the compounds of the invention. In addition to the latter, D-residue are used after each proline amino acid, since it has been reported that a frequent endopeptidase cleavage site is after this residue by an enzyme known as prolylendopeptidase.

Alzheimer's Inhibitors

leu pro phe phe asp

leu Pro Phe Phe asp

leu Pro phe Phe asp

Prion Inhibitors

asp ala pro ala ala pro ala gly pro ala val pro val

asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro val

asp Ala Pro ala Ala Pro ala Gly Pro ala Val Pro val

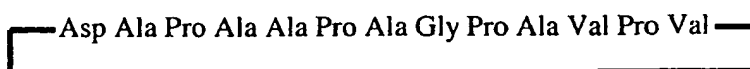
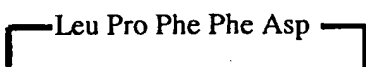
Amino acids written with lower case letters denote D-residue.

e) *Cyclic peptides*. Conformationally constrained cyclic peptides represent better drug candidates than linear peptides due to their reduced conformational flexibility and improved resistance to exopeptidase cleavage. Two alternative strategies have been used to convert a linear sequence into a cyclic structure. One is the introduction of cysteine residue

to achieve cyclization through the formation of a disulfide bridge and the other is the side-chain attachment strategy involving resin-bound head-to-tail cyclization. To avoid modifications of the peptide sequence the latter approach is used. β -sheet breaker peptides contain the ideal sequences for facilitating macrocyclization because proline, due to its ability to promote turns and loops, is a constituent of many naturally occurring or artificially synthesized cyclic peptides.

Alzheimer's Inhibitors

Prion inhibitors



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f) *Pseudopeptides*. Pseudopeptides or amide bond surrogates refers to peptides containing chemical modifications of some (or all) of the peptide bonds. Amide bond replacements are usually represented by retaining the amino acid designation according to the side-chain and specifying the changes that occur between the α -carbons, using the nomenclature known as "psi-bracket."

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For example, the term $\text{Ala}\psi[\text{CH}_2\text{CH}_2]\text{Gly}$ refers to the moiety $\text{NH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$. Several amide bond surrogates have been described in Table 2 below.

Table 2. Some amide bond surrogates and their properties

Surrogate	Properties
CH_2	Short, flexible
CH_2CH_2	Flexible, hydrophobic
$\text{CH} = \text{CH}$	Rigid, hydrophobic
$\text{C} \equiv \text{C}$	Very rigid
$\text{CH}_2 \text{NH}$	Flexible, hydrophilic
COCH_2	Flexible, hydrophilic
$\text{CH}_2 \text{S}$	Flexible, hydrophobic
$\text{CH}_2 \text{SO}_2$	More rigid, hydrophilic
NHCO	Rigid, hydrophilic

Some of them are found in naturally occurring peptide analogs (such as $\psi[\text{CHOH}]$, $\psi[\text{CSNH}]$, $\psi[\text{COO}]$) while others have been artificially synthesized. The introduction of amide bond surrogates not only decreases peptide degradation but also may significantly modify some of the biochemical properties of the peptides, particularly the conformational flexibility and hydrophobicity. It is likely that an increase in conformational flexibility will be beneficial for docking the inhibitor to the A β and PrP binding sites. On the other hand, since the interaction between the amyloidogenic proteins and the inhibitors seems to depend to a great extent on hydrophobic interactions, it is likely that amide bond replacement increasing hydrophobicity may enhance affinity and hence, potency of the inhibitors. In

addition, increased hydrophobicity could also enhance transport of the peptide across membranes and thus, improve barrier permeability (blood-brain barrier and intestinal barrier). Therefore, to synthesize pseudopeptides amide bond replacement is used thereby increasing flexibility and hydrophobicity, such as $\psi[\text{CH}_2\text{CH}_2]$ and $\psi[\text{CH}_2\text{S}]$. The amide bonds to replace are those located at the end of the peptide to prevent exoprotease degradation and after each of the prolines, since it has been described that a frequent endopeptidase cleavage site occurs after this residue by an enzyme known as prolylendopeptidase. Additional amide bonds that need to be protected are determined by experimental studies involving the analysis of the degradation of β -sheet breaker peptides in the plasma and tissue.

Alzheimer's Inhibitors

Leu $\psi[\text{CH}_2\text{CH}_2]$ Pro $\psi[\text{CH}_2\text{CH}_2]$ Phe Phe $\psi[\text{CH}_2\text{CH}_2]$ Asp

Leu $\psi[\text{CH}_2\text{S}]$ Pro $\psi[\text{CH}_2\text{S}]$ Phe Phe $\psi[\text{CH}_2\text{S}]$ Asp

Prion inhibitors

Asp $\psi[\text{CH}_2\text{CH}_2]$ Ala Pro $\psi[\text{CH}_2\text{CH}_2]$ Ala Ala Pro $\psi[\text{CH}_2\text{CH}_2]$ Ala Gly Pro $\psi[\text{CH}_2\text{CH}_2]$ Ala Val Pro $\psi[\text{CH}_2\text{CH}_2]$ Val
Asp $\psi[\text{CH}_2\text{S}]$ Ala Pro $\psi[\text{CH}_2\text{S}]$ Ala Ala Pro $\psi[\text{CH}_2\text{S}]$ Ala Gly Pro $\psi[\text{CH}_2\text{S}]$ Ala Val Pro $\psi[\text{CH}_2\text{S}]$ Val

g) Mixture of several modifications. By taking into account the features of the peptide drugs on the market or under current development, it is clear that most of the peptides successfully stabilized against proteolysis consist of a mixture of several types of the above described modifications. This conclusion makes sense in the light of the knowledge that many different enzymes are implicated in peptide degradation. The

following structures contain combinations of different types of chemical modifications:

Alzheimer's Inhibitors

Ac-Leu Proψ[CH₂CH₂]Phe Phe Asp-Am

Ac-Leu Proψ[CH₂S]Phe Phe Asp-Am

5 (Me)Leu Proψ[CH₂CH₂]Phe Phe Asp-Am

leu Proψ[CH₂CH₂]Phe Phe asp

leu Proψ[CH₂S]Phe Phe asp

Ac-Leu Aib Phe Phe Asp-Am

(Me)Leu Aib Phe Phe Asp-Am

10 Leu Proψ[CH₂CH₂]Phe Phe asp

Leu Aib Phe Phe Asp

Leu Proψ [CH₂CH₂] Phe Phe Asp

15 Ac-Leu pro Phe Phe Asp-Am

Ac-Leu Proψ[CH₂CH₂]Phe phe Asp-Am

Ac-Leu Proψ[CH₂S]Phe phe Asp-Am

Ac-Leu Proψ[CH₂CH₂]Phe (Me)Phe Asp-Am

Ac-Leu Proψ[CH₂CH₂]Phe (Me)Phe asp

20 Ac-Leu Pro phe phe Asp-Am

Ac-Leu Pro (Me)Phe phe Asp-Am

leu Proψ[CH₂CH₂]Phe phe asp

leu Pro (Me)Phe phe asp

Ac-Leu Aib Phe phe Asp-Am

25 Prion inhibitors

Ac-Asp Ala Proψ[CH₂CH₂]Ala Ala Proψ[CH₂CH₂]Ala Gly Proψ[CH₂CH₂]Ala Val Pro Val Val

asp Ala Proψ[CH₂CH₂]Ala Ala Proψ[CH₂CH₂]Ala Gly Proψ[CH₂CH₂]Ala Val Pro val

Ac-Asp Ala Proψ[CH₂S]Ala Ala Proψ[CH₂S]Ala Gly Proψ[CH₂S]Ala Val Pro Val-Am

asp Ala Proψ[CH₂S]Ala Ala Proψ[CH₂S]Ala Gly Proψ[CH₂S]Ala Val Pro val

5 Ac-Asp Ala Aib Ala Ala Aib Ala Gly Aib Ala Val Pro Val-Am

Ac-Asp Ala Proψ[CH₂CH₂]Ala Ala Proψ[CH₂CH₂]Ala Gly Proψ[CH₂CH₂]Ala Val Pro (Me)Val

Ac-Asp Ala pro Ala Ala Proψ[CH₂CH₂]Ala Gly pro Ala Val Pro Val-Am

asp Ala Proψ[CH₂CH₂]Ala Ala Proψ[CH₂CH₂]Ala Gly Proψ[CH₂CH₂]Ala Val Pro (Me)Val

asp Ala Aib Ala Ala Proψ[CH₂CH₂]Ala Gly pro Ala Val Pro (Me)Val

10 asp Ala Aib Ala Ala Proψ[CH₂S]Ala Gly pro Ala Val Pro (Me)Val

asp Ala Proψ[CH₂S]Ala Ala Proψ[CH₂S]Ala Gly Proψ[CH₂S]Ala Val Pro (Me)Val

Ac-Asp Ala Aib Ala Ala Proψ[CH₂CH₂]Ala Gly Aib Ala Val Pro (Me)Val

Asp Ala pro Ala Ala Proψ[CH₂CH₂] Ala Gly pro Ala Val Pro Val

15

Asp Al Aib Ala Ala Proψ[CH₂ CH₂] Ala Gly Aib Ala (Me) Val Pro Val

Ac-Asp Ala Proψ[CH₂S]Ala ala Proψ[CH₂S]Ala gly Proψ[CH₂S]Ala (Me)Val Pro Val-Am

Ac-Asp Ala Aib ala Ala Proψ[CH₂CH₂]Ala Gly pro Ala Val Pro (Me)Val

asp Ala Aib Ala Ala Proψ[CH₂CH₂]Ala Gly Aib ala Val Pro Val-Am

20 Ac-Asp Ala pro Ala Ala Proψ[CH₂CH₂]Ala gly pro Ala (Me)Val Pro Val-Am

asp Ala Proψ[CH₂CH₂]Ala Ala Proψ[CH₂CH₂]Ala gly Proψ[CH₂CH₂]Ala val Pro val

Ac-Asp Ala pro Ala ala Aib Ala gly pro Ala (Me)Val Pro Val-Am

Asp Ala pro Ala Ala Proψ[CH₂ CH₂] Ala Gly pro Ala Val Pro Val

Asp Ala Aib Ala Ala Proψ[CH₂ CH₂] Ala Gly Aib Ala (Me) Val Pro Val

Another approach to improve stability, which also may result in the generation of orally active compounds, is to produce a peptide mimetic. A peptide mimetic is a molecule that mimics the biological activity of the peptides, but is no longer a peptide in chemical nature. The term peptide mimetic has been used sometimes to describe molecules that are partially peptide in nature, such as pseudopeptides, semi-peptides or peptoids, but a strict definition and the one that is used in the present application is an organic molecule that no longer contains any peptide bonds. Peptide mimetics are not derivatives of a parent peptide, but rather are chemically synthesized *de novo* trying to mimic the structural and functional properties of the peptide. The rational design of peptide mimetics requires a sufficient knowledge of the pharmacophoric groups that are responsible for the activity and detailed structural information of the peptide. The objective is to reconstruct the spatial position of the pharmaco-active groups using an organic template to mount them. Selection of the template is important and has to take into consideration the size and flexibility based on the conformational model of the peptide.

Peptide mimetics designed to imitate β -sheet breaker peptide properties.

The rational design of peptide mimetics requires a sufficient knowledge of the chemical groups that are responsible for the activity and detailed structural information of the peptide. The objective is to reconstruct the position of the pharmaco-active groups using an organic template to mount them. Selection of the template is important and has to take

into consideration the size and flexibility based on the conformational model of the peptide.

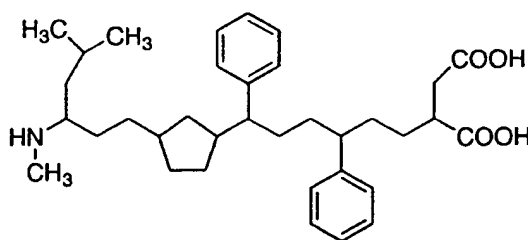
From the study of the activity of different β -sheet breaker sequences bearing single amino acid substitutions, the residues that are key for inhibition have been determined. In addition, the tridimensional structure of the lead Alzheimer's and prion β -sheet breaker peptides

5 (FIGS. 3A and 3B) were either modeled or experimentally determined. The 5-residue inhibitor of A β fibrillogenesis was modeled by energy minimization and Monte Carlo simulations using the computer program ICM. The structure of the 13-residue inhibitor of prion protein conformational changes was experimentally calculated by 2D-NMR.

There are numerous approaches to the design and synthesis of peptide mimetics as
10 described in recent reviews by Joachim Gante and Iwao Ojima et al. of which are incorporated herein by reference.

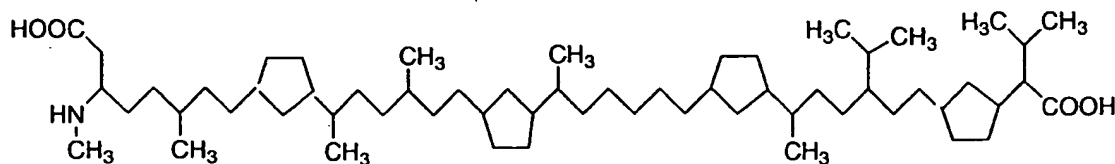
The peptide mimetics shown below represent a further aspect of this invention.

Alzheimer's Inhibitors

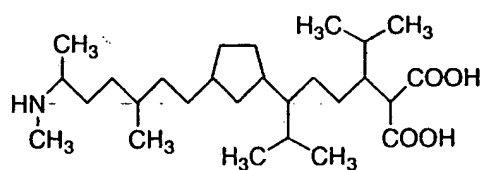


15

PMiA85

Prion inhibitors

PMiPrP13



PMiPrP5

The latter (PMiPrP5) is a shorter and easier to synthesize version that contains the chemically active groups and is analog to a 5-residue prion β -sheet breaker peptide.

As a method of preventing or treating a disorder or disease associated with amyloid or amyloid-like deposits or pathological beta-sheet-rich precursors thereof, the compound of the present invention is administered in an effective amount to a subject in need thereof, where the subject can be human or animal. Likewise, a method of detecting such disorders or diseases also includes administering a sufficient amount of the designed compound to visualize its binding to fibril deposits or precursors thereof by well-known imaging techniques.

As used herein, the term "prevention" of a condition, such as Alzheimer's disease or other amyloidosis disorders, in a subject involves administering the compound according to the present invention prior to the clinical onset of the disease. "Treatment" involves administration of the protective compound after the clinical onset of the disease. For example, successful administration of the compound of the present invention, after development of a disorder or disease comprises "treatment" of the disease. The invention is useful in the treatment of humans as well as for veterinary uses in animals.

The compound of the present invention may be administered by any means that achieves its intended purpose, preferably oral. For example, administration may be by a number of different parenteral routes including, but not limited to, subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intracerebral, intranasal, oral, transdermal, or buccal routes. Parenteral administration can be bolus injection or by gradual perfusion over time.

A typical regimen for preventing, suppressing, or treating a condition associated with amyloid or amyloid-like deposits, comprises either: (1) administration of an effective amount in one or two doses of a high concentration of the compound in the range of 0.5 to 10 mg, more preferably 0.5 to 5 mg, or (2) administration of an effective amount of the compound administered in multiple doses of lower concentrations in the range of 10 – 10,000 µg, more preferably 50 – 500 µg over a period of time up to and including several months to several years.

It is understood that the dosage administered will be dependent upon the age, sex,

health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The total dose required for each treatment may be administered by multiple doses or in a single dose. By "effective amount," it is meant a concentration of the compound which is capable of slowing down or inhibiting the formation of amyloid or amyloid-like deposits, or pathological beta-sheet precursors thereof, or of dissolving preformed fibril deposits. Such concentrations can be routinely determined by those of skill in the art. It will also be appreciated by those of skill in the art that the dosage may be dependent on the stability of the administered compound. A less stable compound may required administration in multiple doses.

10 Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain auxiliary agents or excipients which are known in the art. Pharmaceutical compositions such as tablets and capsules can also be prepared according to routine methods.

15 Pharmaceutical compositions comprising the compound of the invention include all compositions wherein the compound is contained in an amount effective to achieve its intended purpose. In addition, the pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Suitable pharmaceutically acceptable vehicles are well known in the art and are described for example in Gennaro, Alfonso, Ed., *Remington's Pharmaceutical Sciences*, 18th Edition
20 1990, Mack Publishing Co., Easton, PA, a standard reference text in this field.

Pharmaceutically acceptable vehicles can be routinely selected in accordance with the mode of administration and the solubility and stability of the compound. For example, formulations for intravenous administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

5 Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspension of the active compound as appropriate oily injections suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example ethyl oleate or triglycerides. Aqueous injection
10 suspensions that may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

Disorders or diseases associated with abnormal protein folding into amyloid or amyloid-like deposits or into pathological beta-sheet-rich precursors of such deposits to be
15 treated or prevented by administering the pharmaceutical composition of the invention includes, but is not limited to, Alzheimer's disease, FAF, Down's syndrome, other amyloidosis disorders, human prion diseases, such as kuru, Creutzfeldt-Jakob Disease (CJD), Gerstmann-Strauslet-Scheinker Syndrome (GSS), prion associated human neurodegenerative diseases as well as animal prion diseases such as scrapie, spongiform
20 encephalopathy, transmissible mink encephalopathy and chronic wasting disease of mule deer and elk.

EXAMPLES

One of the major drawbacks for the use of peptides as drugs is their rapid proteolytic degradation in biological fluids and tissues. In *in vitro* experiments, iA β 5, (Seq LPFFD- also depicted as Leu Pro Phe Phe Asp herein) degraded very quickly in vitro after incubation with fresh human plasma. As shown in Figure 4a, fifty percent of the peptide iA β 5 disappeared in approximately 5 minutes in the presence of plasma. Since it was not possible to identify any metabolic fragments as a result of the proteolytic digestion, it seems likely that the degradation is mainly done by unspecific exopeptidases. This conclusion is supported by the finding that protection of amino- and carboxy-terminus of the peptide by acetylation and amidation, respectively, (to form Ac-iA β 5-Am -- also depicted as Ac-Leu Pro Phe Phe Asp-Am herein) dramatically increases the stability of the peptide *in vitro*. As shown in Fig. 4b, the end-protected modified peptide of the present invention (Ac-iA β 5-Am) remained stable for a period of more than 24 hours in human plasma. (The modified peptide was also slowly metabolized in vitro in human and rat liver microsomes, in which after one hour of incubation at 37° a 81.5% and 76.3% of the peptide remained intact, in human and rat tissue homogenate, respectively.)

Additional *in vitro* studies showed that Ac-iA β 5-Am has similar activity as iA β 5 in inhibiting amyloid formation (see Fig. 5a) and the effect followed a similar dose-dependency as the activity of the unmodified peptide as shown in Figures Fig. 5b and 5c. Returning to Fig. 5a, it can be seen that modification of the N-terminus by Boc also retains the *in vitro*

activity exhibited by iA β 5 while several unrelated peptides (CP1:VHVSEEGTET A, CP2:GYLTVA AVFRG, CP10: ISEVKMDAEF) or short A β fragments (such as A β 18-21, A β 1-16) at the same concentrations had no effect on fibrillogenesis or slightly increased amyloid formation probably by incorporation into the fibrils.

5

To evaluate the effect of Ac-iA β 5-Am *in vivo*, we used a rat model in which amyloidosis was induced by intracerebral injection of non-aggregated A β 1-42. After some time, the peptide aggregates inside the rat brain resulting in the formation of a single amyloid-like deposit in the place of injection. These lesions have the same tinctorial (congo
10 red birefringence and thioflavine S binding) and translucent (fibrillar structure under electron microscopy) properties than Alzheimer's amyloid plaques and induce some cerebral damage similar to that observed in AD brain, including extensive neuronal shrinkage, astrogliosis and microglial activation. Using this model, we have shown previously that co-injection of the unprotected iA β 5 with A β 1-42 induce a 50% inhibition of amyloid plaque formation and
15 i. c. injection of iA β 5 in animals already containing amyloid plaques produced a 67% dissolution of preformed deposits. (Sigurdsson, E.M., Permann, B. Soto, C. , Wisniewski, T. & Frangione, B. (2000) In vivo disassembly of amyloid- β deposits in rat brain. *J. Neuropath. Exp. Neurol.* 59: 11-17) In the previous experiments, the unprotected peptide was injected directly in the brain region where the amyloid was located. In the present
20 experiment the amyloid- β 5 peptide was injected into the amygdala of the rats. After 7 days, which is the time required to have fully formed the amyloid deposits, one-hundred μ L

of a solution containing 13 mg/ml of the Ac-iA β 5-Am were infused for a period of three weeks using an ALZET infusion pump connected to the lateral ventricle. The animals were sacrificed and the brain analyzed for the presence of amyloid deposits by immunohistochemistry. In this model, a compacted amyloid plaque was obtained in the place where the solution containing A β 1-42 was deposited (amygdala) and also several smaller amyloid deposits were observed throughout the canula track in regions closer to the ventricle (Fig. 6, left panel). The results show that infusion of the peptide induces a 30% dissolution of preformed amyloid plaque in the amygdala and 83% dissolution of the deposits located near the ventricle (Fig. 6).

10

Experimental Procedures

In vitro assays of peptide stability. Peptides were prepared as a 1 $\mu\text{g}/\mu\text{l}$ solution in water. 20 μl of the peptide solution was diluted in 80 μl of fresh human plasma. The solution was incubated at 37°C for different time periods and the reaction was stopped by adding a complete cocktail of protease inhibitors. The bulk of the plasma proteins (none of the peptide) were precipitated in cold methanol (mix/MeOH, 4/5, v/v) for one hour at -20°C. The precipitated proteins were pelleted by centrifugation (10 000g, 10 min, 4°C). The supernatant, containing the peptide, was concentrated 5 times under vacuum and separated by reverse-phase HPLC. The peak area corresponding to the intact peptide was measured and compared with an equivalent sample incubated without plasma.

20

In vitro assays of activity. Amyloid formation was quantitatively evaluated by the fluorescence emission of thioflavine T (ThT) bound to amyloid fibrils. Aliquots of A β at a concentration of 0.5 mg/ml prepared in 0.1M Tris, pH 7.4 were incubated for 7 days at 37°C in the absence or in the presence of different concentrations of iA β 5 and derivatives. At the end of the incubation period, 50 mM glycine, pH 9.2 and 2 μ M ThT were added in a final volume of 2 ml. Fluorescence was measured at: excitation 435 nm and emission 485 nm in a Perkin Elmer, model LS50B fluorescence spectrometer.

In vivo studies using an animal model of cerebral AP deposition. Male Fischer-344 rats weighed 250-300g and were 3-4 months of age at the time of arrival. The animals were housed 2 per cage, maintained on a 12 hour light-dark cycle with access to food and water *ad libitum* and were habituated to their new environment for 2-3 weeks prior to surgery. Surgery was performed under sodium pentobarbital (50 mg/kg, i.p.) anesthesia. Atropine sulfate (0.4 mg/kg) and ampicillin sodium salt (50 mg/kg) were injected subcutaneously once the animals were anesthetized. A β 1-42 was dissolved in dimethylsulfoxide (DMSO) and then diluted with water to a 16.7% DMS. The animal received a bilateral injection of 5.0 nmol A β 1-42 into each amygdala by using a Kopf stereotaxic instrument with the incisor bar set at 3.3 mm below the interaural line. Injection coordinates measured from the bregma and the surface of the skull (AP -3.0, ML \pm 4.6 DV -8.8) were empirically determined based on the atlas of Paxinos and Watson. A volume of 3.0 μ l was administered over 6 min (flow

rate 0.5 μ l/min) using a CMA-100 micrasyringe pump. The cannula was left *in situ* for 2 min following injection, then it was withdrawn 0.2 mm and left for 3 min, and after 5 min the cannula was slowly withdrawn. Following surgery the animals were placed on a heating pad until they regained their righting reflex. To evaluate the effect of Ac-iB5-AM the animals were subjected to a second surgery one week after the first one, in which an ALZET infusion pump was connected to the cerebral ventricle following the manufacturer indications. A total of 1.3 mg of peptide in 100 μ l of PBS/10% DMSO was delivered into the lateral ventricle over a period of 3 weeks. After this time, the animals were sacrificed by an overdose of sodium pentobarbital (150 mg/kg, i.p.), perfused transaortically. For histology, serial coronal sections (40 μ m) of the brain were cut, placed in ethylene glycol cryoprotectant and stored at -20°C until stained. Tissue sections were stained with anti A β 1-42 antibodies as described in Soto, C., Sigursson, E., Morelli, L., Kumar, R.A., Castaño, E.M. and Frangione, B.(1998) β -sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: Implications for Alzheimer's therapy. *Nature med.* 4: 822-826. An image analysis system was used to determine the size of the amyloid deposits. The data was analyzed by a two-way ANOVA followed by a Newman-Keuls' multiple range test for *post hoc* comparisons. Total brain deposition was analyzed using an unpaired t-test, two tailed.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters,

concentrations, and conditions without departing from the spirit and scope of the invention and without due experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without

departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and
5 not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

WHAT IS CLAIMED IS:

1. An inhibitory peptide capable of inhibiting β pleated sheet formation in amyloid β -peptide said inhibitory peptide being a β sheet breaker peptide analog designed by chemical
5 modification of a β sheet breaker peptide capable of inhibiting β pleated sheet formation in amyloid β -peptide.

2. The inhibitory peptide of claim 1 wherein said β sheet breaker peptide is a 5 residue
Alzheimer inhibitor peptide iA β 5 (Seq. Leu-Pro-Phe-Phe-Asp)

10

3. The inhibitory peptide of claim 2 wherein said chemical modification is achieved by
a process selected from the group consisting of: alteration of the N- and C- terminal ends of
said Alzheimer inhibitor peptide iA β 5; replacing at least one residue of said Alzheimer
inhibitor peptide iA β 5 with α -aminoisobutyric acid (Aib); methylation of the α carbon of at
15 least one residue of said Alzheimer inhibitor peptide iA β 5; replacing at least one L-
enantiomeric residue of said Alzheimer inhibitor peptide iA β 5 with a D-enantiomeric
residue, forming head to tail cyclization of said Alzheimer inhibitor peptide iA β 5, replacing
amide bonds in said Alzheimer inhibitor peptide iA β 5 with an amide bond surrogate; and
combinations thereof.

20

4. The inhibitory peptide of claim 3 said alteration of the N- and C- terminal

ends of said Alzheimer inhibitor peptide iAβ5 is achieved by a process selected from acetylation, amidation, desamination, alcoholization and combinations thereof.

5. The compound of Claim 4 wherein inhibitory peptide is selected from the group consisting of: ac-Leu Pro Phe Phe Asp-am, des-Leu Pro Phe Phe Asp-am, ac-Leu Pro Phe Phe Asp-alc, and
5 des-Leu Pro Phe Phe Asp-alc,

6. The inhibitory peptide of Claim 5 wherein said inhibitory peptide is ac-Leu Pro Phe Phe Asp-am.

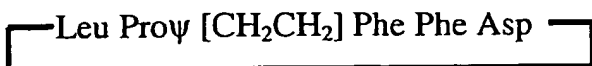
10 7. The inhibitory peptide of claim 3 wherein said inhibitory peptide is selected from the group consisting of Leu Aib Phe Phe Asp; (Me)Leu Pro Phe Phe Asp; Leu Pro (Me)Phe Phe Asp, Leu Pro Phe (Me)Phe Asp; (Me)Leu Pro (Me)Phe (Me)Phe Asp, leu pro phe phe asp, leu Pro Phe Phe asp, leu Pro phe Phe asp,

Leuψ[CH₂CH₂]Proψ[CH₂CH₂]Phe Pheψ[CH₂CH₂]Asp; Leuψ[CH₂S]Proψ[CH₂S]PhePheψ[CH₂S]Asp;

15 Ac-Leu Proψ[CH₂CH₂]Phe Phe Asp-Am;
Ac-Leu Proψ[CH₂S]Phe Phe Asp-Am;
(Me)Leu Proψ[CH₂CH₂]Phe Phe Asp-Am;
leu Proψ[CH₂CH₂]Phe Phe asp;
leu Proψ[CH₂S]Phe Phe asp;

20 Ac-Leu Aib Phe Phe Asp-Am;
(Me)Leu Aib Phe Phe Asp-Am;
Leu Proψ[CH₂CH₂]Phe Phe asp;

Leu Aib Phe Phe Asp



Ac-Leu pro Phe Phe Asp-Am;

5 Ac-Leu Proψ[CH₂CH₂]Phe phe Asp-Am;

Ac-Leu Proψ[CH₂S]Phe phe Asp-Am;

Ac-Leu Proψ[CH₂CH₂]Phe (Me)Phe Asp-Am;

Ac-Leu Proψ[CH₂CH₂]Phe (Me)Phe asp;

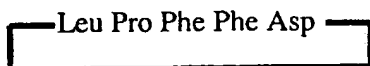
Ac-Leu Pro phe phe Asp-Am;

10 Ac-Leu Pro (Me)Phe phe Asp-Am;

leu Proψ[CH₂CH₂]Phe phe asp;

leu Pro (Me)Phe phe asp;

Ac-Leu Aib Phe phe Asp-Am; and



15

8. An inhibitory peptide capable of inhibiting conformational changes in prion PrP protein associated with amyloidosis, said inhibitory peptide being a βsheet breaker peptide analog designed by chemical modification of a βsheet breaker peptide capable inhibiting said conformational changes in prior PrP protein associated with amyloidosis.

20

9. The inhibitory peptide of claim 8 wherein said βsheet breaker peptide is 13 residue prion inhibitor peptide iPrP13 (Seq. Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro Val).

10. The inhibitory peptide of claim 9 wherein said chemical modification is achieved by a process selected from the group consisting of: alteration of the N- and C- terminal ends of said prion inhibitor peptide iPrP13; replacing at least one residue of said prion inhibitor peptide iPrP13 with α -aminoisobutyric acid (Aib); methylation of the α carbon of at least one residue of said prion inhibitor peptide iPrP13; replacing at least one L-enantiomeric residue of said prion inhibitor peptide iPrP13 with a D-enantiomeric residue, forming head to tail cyclization of said prion inhibitor peptide iPrP13, replacing amide bonds in said prion inhibitor peptide iPrP13 with an amide bond surrogate; and combinations thereof.

11. The inhibitory peptide of claim 10 wherein said alteration of the N- and C-terminal ends of said prion inhibitor peptide iPrP13 is achieved by a process selected from acetylation, amidation, desamination, alcoholization and combinations thereof.

12. The compound of Claim 11 wherein said inhibitory peptide is selected from the group consisting of: ac-Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro Val-am, des-Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro Val-am, ac-Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro Val-alc, and des-Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro Val-alc.

13. The inhibitory peptide of claim 10 wherein said inhibitory peptide is selected from the group consisting of

Asp Ala Aib Ala Ala Aib Ala Ala Aib Ala Gly Aib Ala Val Aib Val;

Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala (Me)Val Pro Val;

Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro (Me)Val;

Asp Ala Pro Ala Ala Pro Ala Gly Pro Ala (Me)Val Pro (Me)Val;

asp ala pro ala ala pro ala gly pro ala val pro val;

asp Ala Pro Ala Ala Pro Ala Gly Pro Ala Val Pro val;

5 asp Ala Pro ala Ala Pro ala Gly Pro ala Val Pro val;

Asp ψ [CH₂CH₂]Ala Pro ψ [CH₂CH₂]Ala Ala Pro ψ [CH₂CH₂]Ala Gly Pro ψ [CH₂CH₂]Ala

ValPro ψ [CH₂CH₂]Val;

Asp ψ [CH₂S]Ala Pro ψ [CH₂S]Ala Ala Pro ψ [CH₂S]Ala Gly Pro ψ [CH₂S]Ala Val Pro ψ [CH₂S]Val,;

Ac-Asp Ala Pro ψ [CH₂CH₂]Ala Ala Pro ψ [CH₂CH₂]Ala Gly Pro ψ [CH₂CH₂]Ala Val Pro Val-Am;

10 asp Ala Pro ψ [CH₂CH₂]Ala Ala Pro ψ [CH₂CH₂]Ala Gly Pro ψ [CH₂CH₂]Ala Val Pro val;

Ac-Asp Ala Pro ψ [CH₂S]Ala Ala Pro ψ [CH₂S]Ala Gly Pro ψ [CH₂S]Ala Val Pro Val-Am;

asp Ala Pro ψ [CH₂S]Ala Ala Pro ψ [CH₂S]Ala Gly Pro ψ [CH₂S]Ala Val Pro val;

Ac-Asp Ala Aib Ala Ala Aib Ala Gly Aib Ala Val Pro Val-Am;

Ac-Asp Ala Pro ψ [CH₂CH₂]Ala Ala Pro ψ [CH₂CH₂]Ala Gly Pro ψ [CH₂CH₂]Ala Val Pro (Me)Val;

15 Ac-Asp Ala pro Ala Ala Pro ψ [CH₂CH₂]Ala Gly pro Ala Val Pro Val-Am;

asp Ala Pro ψ [CH₂CH₂]Ala Ala Pro ψ [CH₂CH₂]Ala Gly Pro ψ [CH₂CH₂]Ala Val Pro (Me)Val;

asp Ala Aib Ala Ala Pro ψ [CH₂CH₂]Ala Gly pro Ala Val Pro (Me)Val;

asp Ala Aib Ala Ala Pro ψ [CH₂S]Ala Gly pro Ala Val Pro (Me)Val;

asp Ala Pro ψ [CH₂S]Ala Ala Pro ψ [CH₂S]Ala Gly Pro ψ [CH₂S]Ala Val Pro (Me)Val;

20 Ac-Asp Ala Aib Ala Ala Pro ψ [CH₂CH₂]Ala Gly Aib Ala Val Pro (Me)Val;

Asp Ala pro Ala Ala Pro ψ [CH₂CH₂] Ala Gly pro Ala Val Pro Val

Asp Al Aib Ala Ala Pro ψ [CH₂ CH₂] Ala Gly Aib Ala (Me) Val Pro Val

25 Ac-Asp Ala Pro ψ [CH₂S]Ala ala Pro ψ [CH₂S]Ala gly Pro ψ [CH₂S]Ala (Me)Val Pro Val-Am;

Ac-Asp Ala Aib ala Ala Proψ[CH₂CH₂]Ala Gly pro Ala Val Pro (Me)Val;

asp Ala Aib Ala Ala Proψ[CH₂CH₂]Ala Gly Aib ala Val Pro Val-Am;

Ac-Asp Ala pro Ala Ala Proψ[CH₂CH₂]Ala gly pro Ala (Me)Val Pro Val-Am;

asp Ala Proψ[CH₂CH₂]Ala Ala Proψ[CH₂CH₂]Ala gly Proψ[CH₂CH₂]Ala val Pro val;

5 Ac-Asp Ala pro Ala ala Aib Ala gly pro Ala (Me)Val Pro Val-Am;

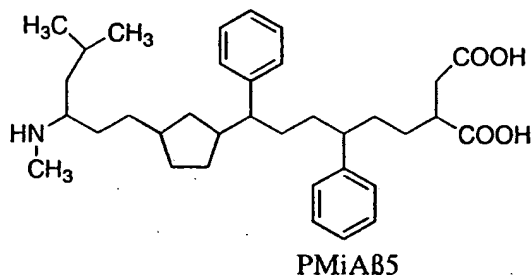
Asp Ala pro Ala Ala Proψ[CH₂CH₂] Ala Gly pro Ala Val Pro Val;

Asp Ala Aib Ala Ala Proψ[CH₂CH₂] Ala Gly Aib Ala (Me) Val Pro Val; and .

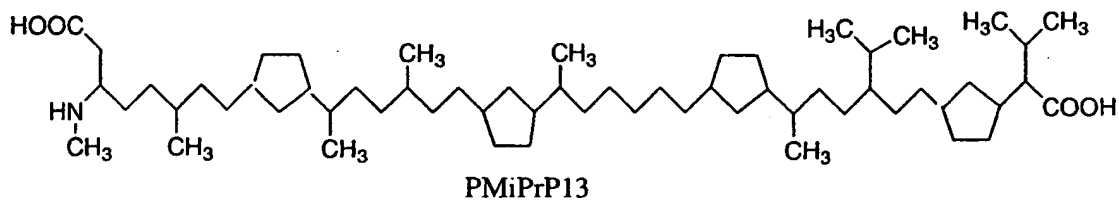
Asp Ala Pro Ala Ala Pro Ala Gly pro Ala Val Pro Val

10

14. A peptide mimetic with the following structure:

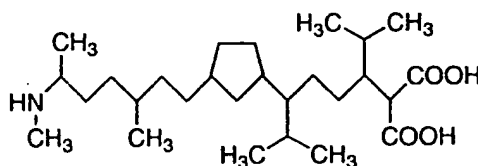


15. A peptide mimetic with the following structure:



15

16. A peptide mimetic with the following structure:

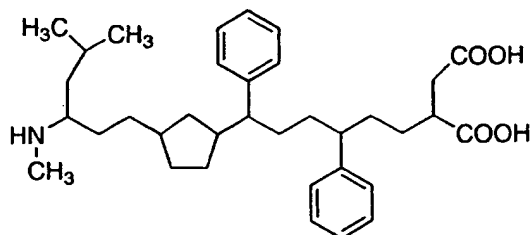


PMiPrP5

17. A method for reducing the formation of amyloid or amyloid like deposits involving abnormal folding into β sheet structure of amyloid β peptide or for reducing the amount of said amyloid β peptide which has already formed into a beta sheet structure comprising bringing into the presence of said amyloid β peptide either prior to or after the abnormal folding thereof into a β sheet structure, an effective amount of the peptide of claim 1.

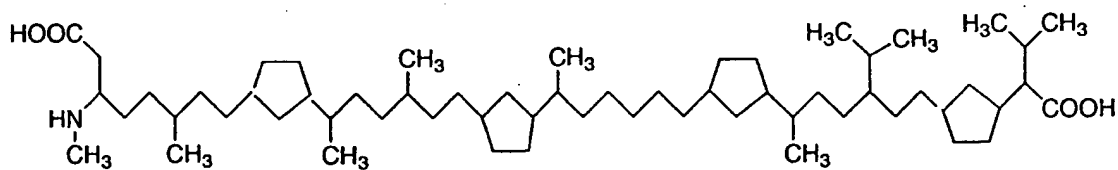
18. A method for reducing the formation of amyloid or amyloid like deposits involving conformational changes in prion Pr protein or reducing the amount of said prion Pr protein which has already formed into amyloid or amyloid-like deposits comprising bringing into the presence of said prion Pr protein either prior to or after said conformational changes thereof into amyloid deposits an effective amount of the peptide of claim 8.

19. A method for reducing the formation of amyloid or amyloid like deposits by administration of a peptide mimetic selected from one of the group consisting of :



5

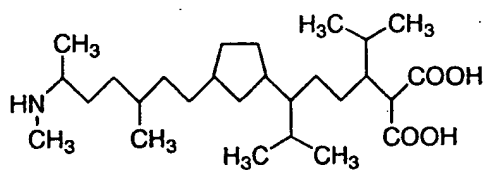
PMiA85,



PMiPrP13

10

and



PMiPrP5

FIG. 1

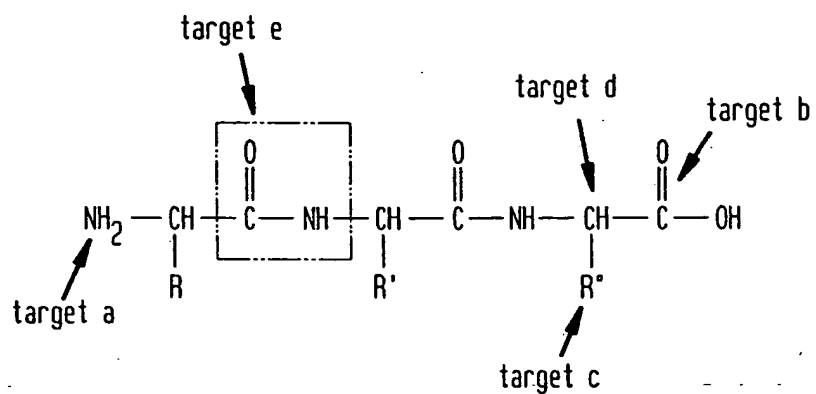
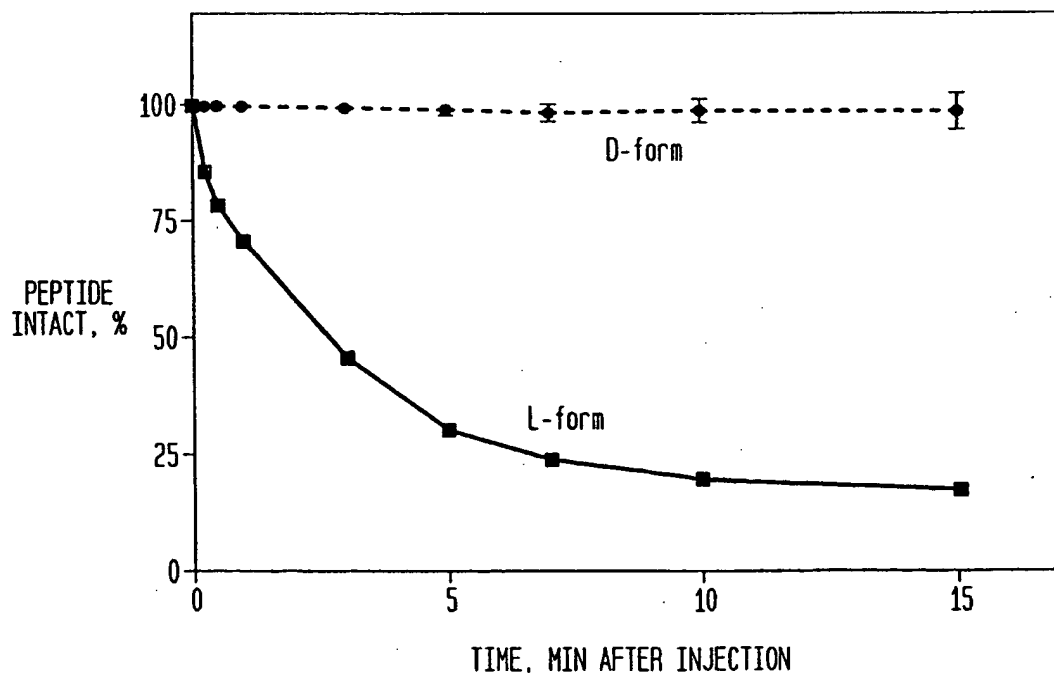


FIG. 2



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FIG. 3A

iA β 5

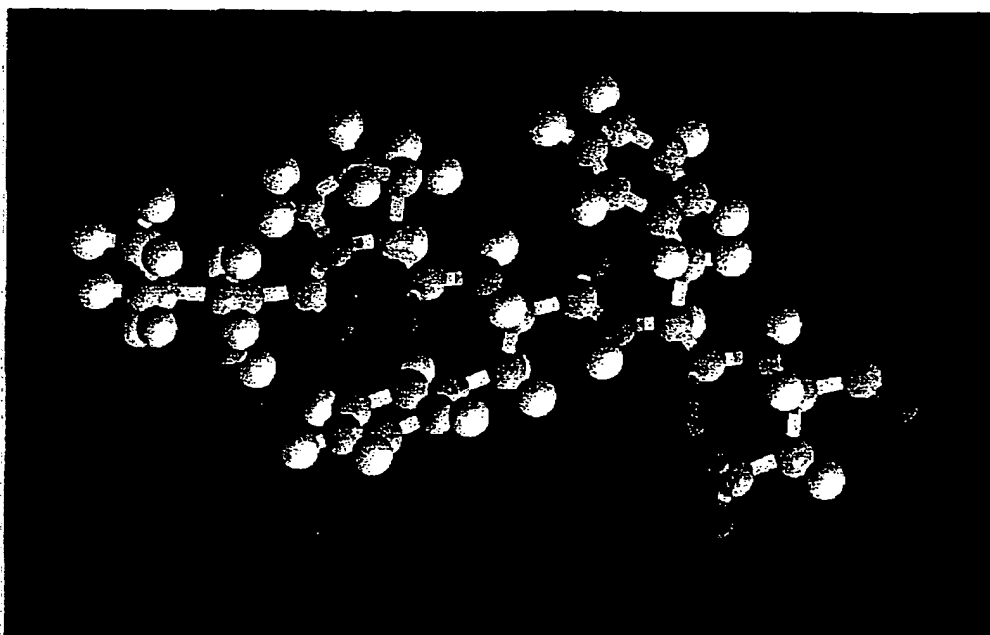
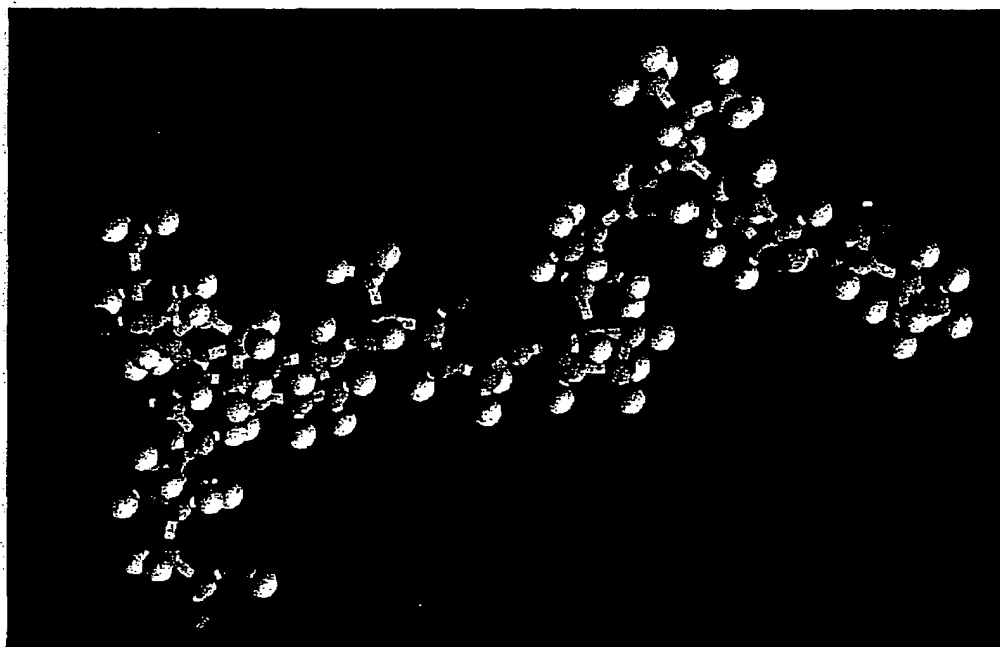


FIG. 3B

iPrP13



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FIG. 4A

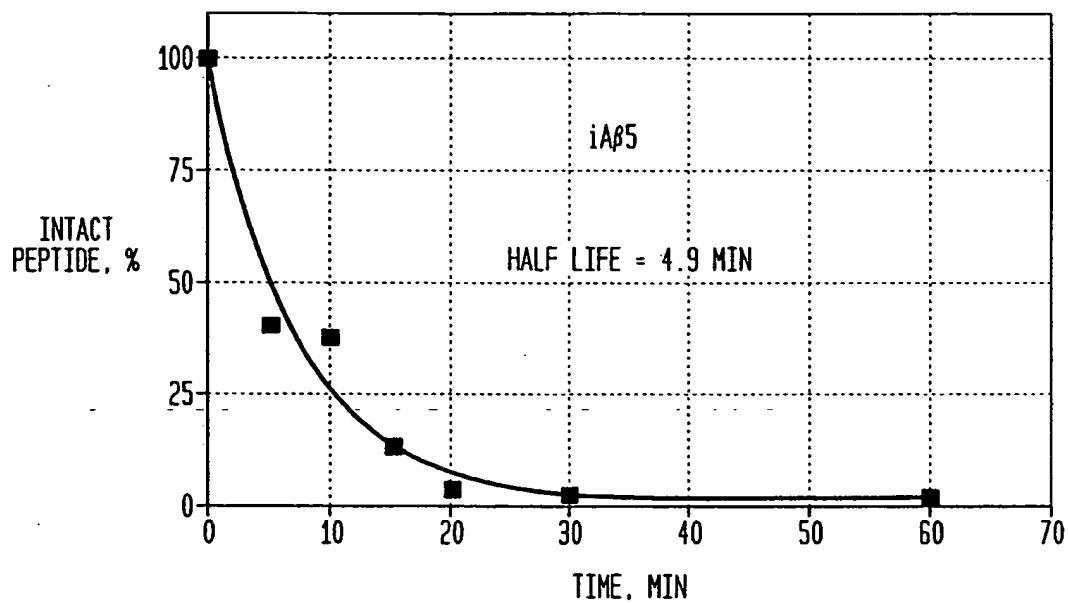


FIG. 4B

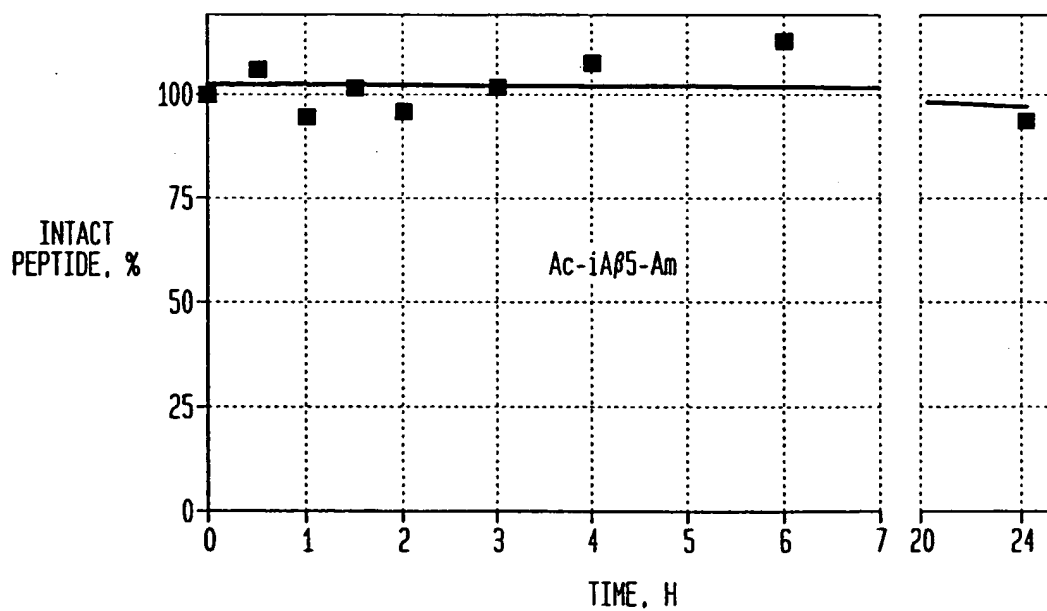
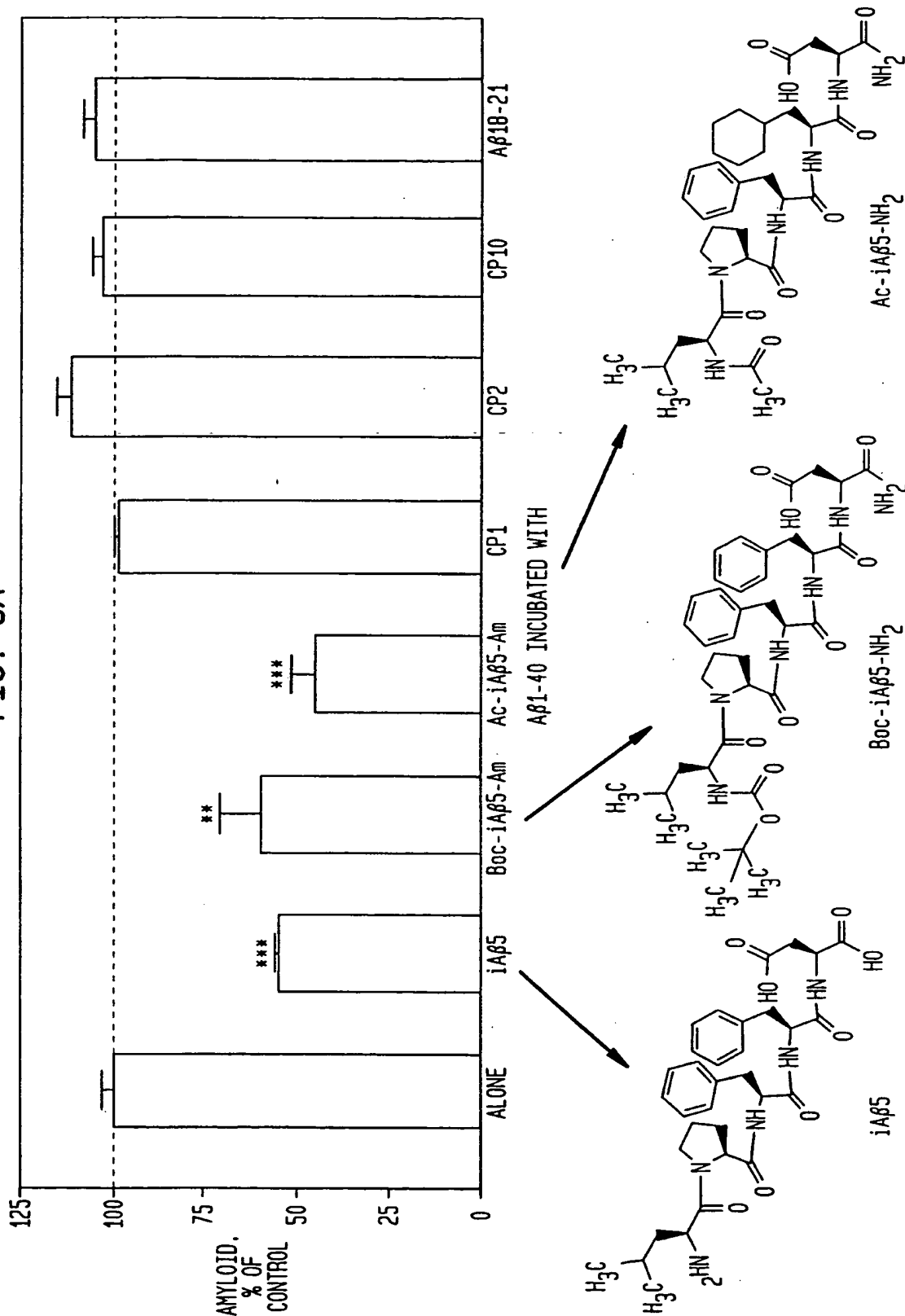


FIG. 5A



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FIG. 5B

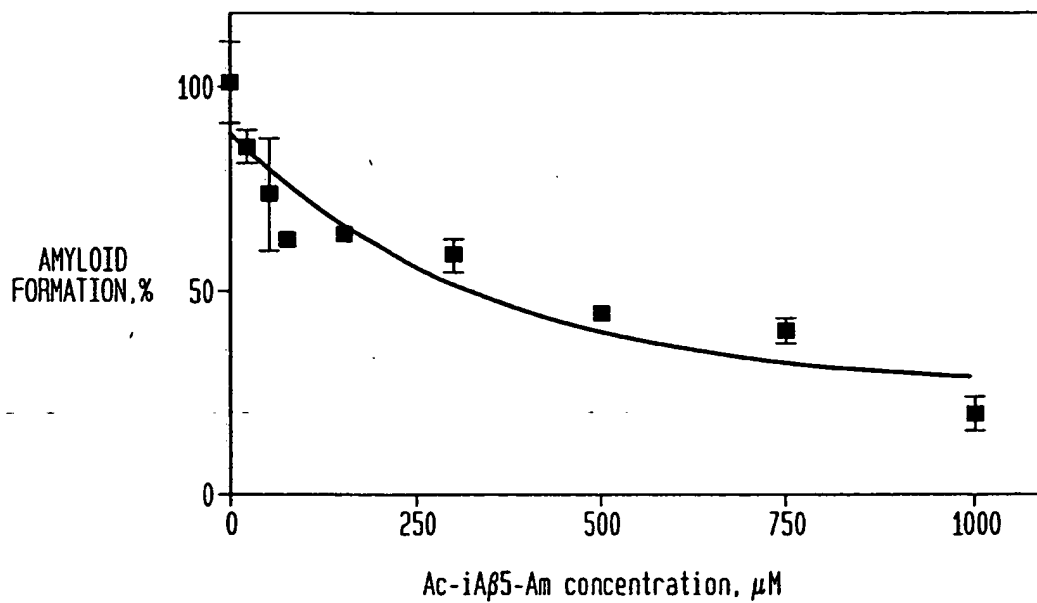
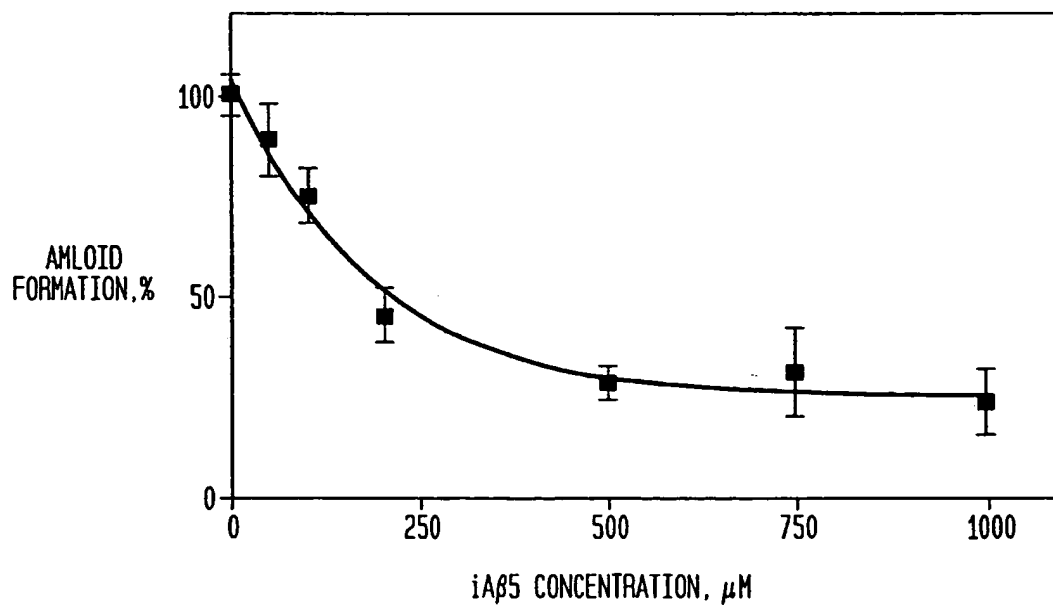
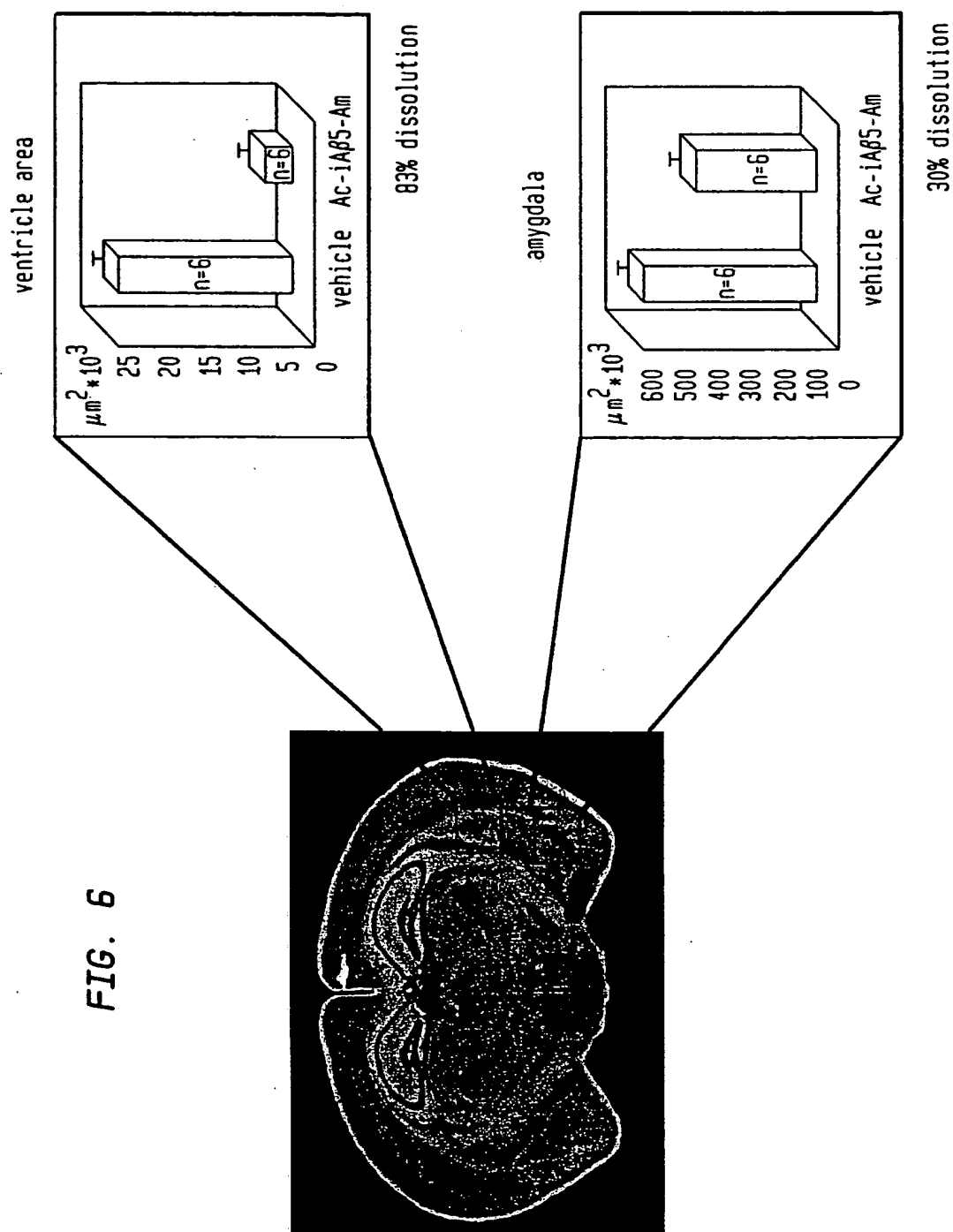


FIG. 5C



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